

WHAT IS CLAIMED IS THE FOLLOWING:

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A1  
1. A method of extracting lipid associated sialoprotein from body fluids such as cerebrospinal fluid, urine, saliva and sputum and determining the amount of lipid associated sialoprotein in a sample of such fluid which comprises the following steps:

a) adding to the sample a mixture of a chlorinated lower alkyl hydrocarbon and a lower alkyl alcohol;

b) mixing the resulting admixture for a suitable period of time to dissolve lipid-bound sialic acid in the sample in the chlorinated hydrocarbon/alcohol mixture;

c) centrifuging the mixture to form a substantially clear upper phase;

d) separately recovering from the clear upper phase so formed a predetermined volume of the upper phase;

e) adding to the predetermined volume of the upper phase an amount of a mixture of an aqueous protein-precipitating agent without any adsorbing material, the amount of mixture being effective to cause precipitation of the lipid associated sialoprotein;

f) vortexing the resulting admixture;

g) separately recovering the resulting precipitate;

h) washing the precipitate in a saline solution;

i) centrifuging the resulting mixture;

j) dissolving the precipitate in water;

k) adding to the solution a hydrolysis agent;  
l) heating the resulting admixture;  
m) determining the amount of lipid associated sialoprotein present in the solution and thereby the amount present in the fluid sample.

2. A method according to claim 1, wherein in step (a) the volume of the added mixture is about 750ul.

3. A method according to claim 1, wherein in step (a) the lower alkyl alcohol is methanol, ethanol, propanol, n-butanol, isopropanol, isobutanol or isoamyl alcohol.

4. A method according to claim 1, wherein in step (a) the chlorinated lower alkyl hydrocarbon is chloroform, methylene chloride or ethylene chloride.

5. A method according to claim 1, wherein in step (b) the mixing takes place for at least 15 seconds.

6. A method according to claim 1, where in step (b) the mixture is centrifuged at about 6000 rpm for at least 5 minutes.

7. A method according to claim 1, wherein in step (d) the separately recovering comprises removing the upper phase from the lower phase.

8. A method according to claim 1, wherein step (d) the predetermined amount of the upper phase is about the same as the volume of the sample.

9. A method according to claim 1, wherein in step (e) the protein-precipitating agent is phosphotungstic acid,

trichloroacetic acid, ammonium sulfate or a mixture thereof.

10. A method according to claim 1, wherein in step (e) the concentration of the protein precipitating agent is between 0.3 and 0.6 milligrams per milliliter.

11. A method according to claim 1, wherein in step (f) the mixing takes place for at least 5 seconds.

12. A method according to claim 1, wherein in step (h), the precipitate is washed with 500ul of a saline solution to remove any trace of contaminants.

13. A method according to claim 1, wherein in step (k) the hydrolysis agent is resorcinol.

14. A method according to claim 1 wherein the step (1) the admixture is heated to a temperature of 115 to 120 degrees centigrade for 15 minutes.

15. A method according to claim 1 wherein step (m) the amount of lipid associated sialoprotein is determined by adding to the suspended precipitate a volume of resorcinol reagent, mixing, boiling for 15 minutes, adding a mixture of butylacetate and n-butanol (85:15 v/v) in a volume about twice said volume of resorcinol reagent mixing, centrifuging for about 5 minutes at above 2500 rpm, separating the organic layer, reading at 580 nm the extracted blue color present in the organic layer, determining the amount of lipid associated sialoprotein by comparing the reading obtained at 580 nm to that obtained for a standard having a known amount of lipid associated sialoprotein

and applying the formula:  $\frac{A \times B}{C}$  Where A=the concentration of lipid associated sialoprotein in the standard, B=the optical density of the sample and C=the optical density of the standard.

16. A method according to claim 15, wherein the volume of resorcinol reagent is about 0.5 ml.

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17. A method of diagnosing cancer in a human subject which comprises determining the amount of lipid associated sialoprotein in a sample of the subject's cerebrospinal fluid, peritoneal fluid, pleural fluid, bronchial washings, saliva or sputum sample according to the method of claim 1 and comparing the amount so determined with values obtained for subjects known to have cancer.

18. A method of diagnosing cancer in a human subject which comprises determining at regular time intervals the amount of lipid associated sialoprotein in a sample of the subject's cerebrospinal fluid, peritoneal fluid, pleural fluid, bronchial washings, saliva or sputum sample according to the method of claim 1 and comparing the amounts so determined with amounts previously obtained for the subject.